ATTACHMENT H

ANNOUNCEMENT OF OPPORTUNITY FOR FEDERAL FUNDING
U.S. ENVIRONMENTAL PROTECTION AGENCY

SCOPES OF WORK

1. Water Quality Monitoring Project for the Florida Keys National Marine Sanctuary

Objectives

The general objective of water quality monitoring is to measure the status and trends of water quality parameters to evaluate progress toward achieving and maintaining water quality standards and protecting and restoring the living marine resources of the Sanctuary. Specific objectives are as follows:

*To provide data needed to make unbiased, statistically rigorous statements about the status and temporal trends of water quality parameters in the Sanctuary as a whole and within defined strata.
*To help define reference conditions in order to develop resource-based water quality standards (biocriteria).
*To provide a framework for testing hypothesized pollutant fate/effect relationships through process-oriented research and monitoring.

Monitoring is defined as the continued observation of Sanctuary waters to determine spatial and temporal variability in water quality. Monitoring involves systematic, long-term data collection and analysis to measure the status of water quality and to detect changes over time. Detecting such changes can focus research on determining the cause, can prompt management decisions for corrective action, and can be used to evaluate the success of corrective action.

Overview

Water quality is monitored using a stratified random design based on a modification of the Sanctuary segmentation framework (Klein and Orlando 1994). In some geographic segments, stations are located along inshore/offshore transects; in others, stations are located randomly within EMAP grid cells. Both approaches meet the requirements of the monitoring program, ie. stations are selected randomly and with equal probability within a segment.

Each water quality station is visited during quarterly surveys. Profiles of temperature, salinity, dissolved oxygen (DO), photosynthetically available radiation (PAR), and in situ fluorescence is performed at each station. Water samples are collected using a Niskin sampler and analyzed for turbidity, nutrient content, and biological parameters. Nutrient parameters analyzed are dissolved ammonium (NH_4), dissolved nitrate + nitrite (NO_x), dissolved nitrite (NO_2), dissolved silicate (SiO_2), total nitrogen (TN), soluble reactive phosphate (SRP), total phosphorus (TP), and total organic carbon (TOC). The biological parameters measured are chlorophyll a (Chl a) and alkaline phosphatase activity (APA). Some parameters are not be measured directly, but

calculated by difference. Nitrate (NO_3) is calculated as NO_x - NO_2. Total inorganic nitrogen (TIN) is calculated as NO_x + NH_4. Total organic nitrogen (TON) is defined as TN - TIN. The light extinction coefficient (k_d in m^{-1}) is calculated as a log function from PAR measurements through the water column.

The Sanctuary water quality monitoring program complements and is coordinated with water quality monitoring programs in adjacent areas. The principal investigator for Sanctuary water quality monitoring is also conducting monitoring programs in Florida Bay, Whitewater Bay, the Big Cypress Swamp, and the Southwest Florida Shelf and has provided information about those programs. The Dade County Department of Environmental Management was contacted for information about water quality monitoring in Biscayne Bay. Station locations in the Sanctuary were selected to minimize overlap, and parameters and methods were chosen to provide comparable data. The Technical Advisory Committee for the Water Quality Protection Program and the Interagency Working Group on Florida Bay provide mechanisms for ensuring future coordination of monitoring.

Monitoring Locations

A modification of the segmentation framework developed by Klein and Orlando (1994) was used to stratify station locations. Figure 1 (available upon request) shows stations plotted on the Sanctuary map (precise locations have been plotted on nautical charts). There will be no sampling in Segment 8 or Florida Bay because the effort would overlap with water quality monitoring activities under an existing Everglades National Park program.

Two different approaches were used to position stations within segments; both meet the criterion of selecting sites randomly and with equal probability. Within Segments 1, 2, 3, 4, and 6, stations were located randomly within EMAP grid cells. Within Segments 5, 7, and 9, stations were located along transects extending from the inshore zone, across Hawk Channel, to the offshore (or reef tract) zone. The EMAP approach could have been used throughout the Sanctuary, however, a transect approach was chosen for Segments 5, 7, and 9 because the Technical Advisory Committee expressed strong interest in sampling across the inshore/offshore gradient on the Atlantic side of the Keys.

Along each transect, one station was positioned randomly within each of three zones (nearshore, Hawk Channel, and offshore). Average distances from shore to the inner and outer edges of Hawk Channel were estimated for each segment based on nautical charts. For stations along a given transect, distances from shore were randomly selected within the three intervals (shoreline to inner edge of Hawk Channel, within Hawk Channel, and outer edge of Hawk Channel to Sanctuary boundary).

At the request of the National Park Service, six sites were added within Dry Tortugas National Park. These are considered as being within Segment 1 of the FKNMS.

To aid in the interpretation of the seagrass and coral reef/hard bottom monitoring data, it was

desirable to co-locate water quality and biological monitoring stations. Each permanent seagrass monitoring station will be located at or near a water quality monitoring station. However, coral reef/hard bottom sites were located independently; some are near water quality stations and others are not. Therefore, it was necessary to add water quality stations at some coral reef/hard bottom monitoring sites. There are a total of 43 coral reef/hard bottom sites.

Parameters and Methods

Each station is sampled quarterly. Due to sample holding time requirements and the large geographic area covered, the sampling effort will not be synoptic even within a segment. However, transects or groups of transects will be sampled within a day and all transects and stations within a segment will be sampled within a few days. Barring weather and logistical problems, the field effort for each quarterly survey is expected to be completed within 20 working days.

The suite of water column parameters to be measured at each station is listed in Table 1 (available upon request). The principal investigator will observe the protocols described in the Phase II report (EPA 1993). The principal investigator will maintain and document all field and analytical protocols used in this project that satisfy the QA/QC requirements described below.

Field Collections and Measurements - Sampling is conducted in Segments 3-9 from small boats, whereas sampling in Segments 1 and 2 requires a larger vessel with facilities for sample processing and analysis on board. Sampling platforms are equipped to satisfy the technical and safety requirements of the project. Sampling stations are located using Global Positioning System (GPS) navigation on each survey. Upon completion of the first survey, the principal investigator produced a summary map of the monitoring station network with a listing of stations names, GPS coordinates, water depths, and bottom type.

A multi-sensor, water quality monitoring instrument (SeaBird CTD) is used to measure physicochemical parameters in the field. Semi-continuous measurements are made throughout the water column using the Seabird CTD in an effort to generate a depth profile of each parameter. The physicochemical parameters measured include depth, salinity, temperature, DO, turbidity, PAR, and in situ fluorescence as described in EPA (1993). The light extinction coefficient (k in m^{-1}) is calculated as a log function from PAR measurements through the water column.

Water samples are collected using a Niskin sampler and analyzed for nutrients, turbidity, and biological parameters. In general, where station depth is <3 m, samples are collected at 0.5 m below the surface. At stations >3 m in depth, samples are collected from 0.5 m below the surface and 1 m above the bottom. QC procedures necessary for ensuring the collection of representative and uncontaminated samples are observed (cleaning water samplers, rinsing sample bottles, minimizing contact of samples with air, etc.).

Laboratory Analyses - Nutrient parameters analyzed are NH_4, NO_x, NO_2, TN, SRP, TP, TOC, and SiO_2. The biological parameters measured are Chl a and APA. Some parameters are not measured directly, but calculated by difference. Nitrate (NO_3) is calculated as NO_X - NO_2. Total inorganic nitrogen (TIN) is calculated as NO_X + NH_4. Total organic nitrogen (TON) is defined as TN - TIN.

Dissolved nutrients are defined using Whatman GF/F filters with a nominal pore size of 0.8 m. A 60 ml sample is collected from a Niskin bottle using a syringe and filtered through a 25 mm Whatman GF/F filter. The filtrate is collected in a 60 ml high density polyethylene (HDPE) bottle and the filter stored in a vial with acetone for extraction of Chl a (surface sample only). An additional 60 ml sample is collected directly from the Niskin bottle for analysis of TN, TP, turbidity, and APA.

NH_4 is analyzed by the indophenol method (Koroleff 1983). NO_2 is analyzed using the diazo method and NO_x is measured as nitrite after cadmium reduction (Grassoff 1983a,b). The ascorbic acid/molybdate method is used to determine SRP (Murphy and Riley 1962). High temperature combustion and high temperature digestion is used to measure TN utilizing a Shimadzu TOC-V (Walsh 1989; Sharp et al. 2002; 2003) and TP (Solórzano and Sharp 1980), respectively. TOC is determined using the high temperature combustion method of Sugimura and Suzuki (1988). Silicate is measured using the heteropoly blue method (APHA 1995). Detailed protocols are presented in EPA (1993).

Samples are analyzed for Chl a content by spectrofluorometry of acetone extracts (Yentsch and Menzel 1963). The degree of P limitation in the water column is estimated using an assay of APA. Protocols are presented in EPA (1993) and elsewhere as noted.

Quality Assurance/Quality Control

The principal investigator will establish a QA Program for water quality monitoring to ensure that the data generated are accurate and representative of actual conditions and that the degree of certainty of the data can be established. In accordance with EPA policy, the Sanctuary water quality monitoring program will adhere to existing rules and regulations governing QA and QC procedures as described in EPA guidance documents. The principal investigator will consult with the EPA Region IV QA/QC Officer on any issues involving QA/QC matters.

The principal investigator will produce and submit a Work/Quality Assurance Project Plan to EPA. Approval of the Plan is required before work can begin and any data can be accepted. Through the Plan, the principal investigator explicitly commits to incorporating procedures that will reduce and maintain random and systematic errors within specified tolerable limits. In addition, the principal investigator will document QC procedures and evaluate the quality of the data being produced. Plans should include or refer to a description of safety, training, and equipment maintenance. Data quality objectives will be developed to ensure the utility of data for the application.

The Work/Quality Assurance Project Plan will be prepared according to the format prescribed in EPA Requirements for Quality Assurance Project Plans for Environmental Data Operations, EPA QA/R-5. The principal investigator should develop the Plan in close coordination with the EPA Region IV QA Officer to minimize delays in the process. The Handbook for Analytical Quality Control in Water and Wastewater Laboratories (EPA 1979) should be consulted for guidance on QC procedures for participating laboratories.

Data Management

The principal investigator will develop and maintain protocols and procedures under a data management program for water quality monitoring to ensure that the data generated are accessible to potential users in a timely manner. All original and ancillary data produced under this project will be generated, processed, stored, and archived in a manner that provides detailed documentation of the procedures used at all stages of data collection, reduction, processing, analysis, and storage.

Under a cooperative agreement with EPA, FMRI developed a data management plan and prototype data management system for the monitoring and research programs. The principal investigator will work with FMRI to identify priority data needs, define data entry formats and QA/QC protocols, and resolve data management conventions and issues (e.g., station nomenclature and codes, parameter codes, the geographic datum, missing number codes, error flags).

The principal investigator will design and develop a computerized database under a commercially/commonly available personal computer based database program with guidance from EPA and FMRI. The database will be designed to contain the original data generated by the project and any ancillary information necessary for interpretation of the data. The database will be developed in a format that will allow the database to be directly imported into the data management system to be implemented by FMRI.

Reporting

The principal investigator will produce a station map, quarterly data reports, and an annual report. The principal investigator will be responsible for ensuring that results are compiled and the complete data set is submitted in a timely fashion to FMRI for inclusion into the Sanctuary database.

Station Map and Coordinates - Upon completion of the first survey, the principal investigator will produce a summary map of the monitoring station network with a listing of station names, GPS coordinates, water depths, and bottom types.

Quarterly Data Reports - Upon completion of the analysis of samples from each quarterly survey, the principal investigator will produce a statistical summary of the data in a logical format based on the segmentation scheme and station design. The statistical summary will include calculated

averages, sample variances, ranges, and number of samples. When appropriate, the principal investigator will provide the summaries in a graphical format. The principal investigator will submit a data and narrative report documenting the results of each quarterly survey. The data report will include the raw data and statistical summaries in hard copy and on disk. The investigator will evaluate the data in accordance with the data quality objectives developed in the Work/Quality Assurance Project Plan.

Annual Report - After completion of analysis of samples from the fourth quarterly survey, the principal investigator will produce statistical summaries of the data collected at each water quality monitoring station to be incorporated in an annual report. All data will be evaluated in relation to the data quality objectives developed in the Work/Quality Assurance Project Plan. The data will be analyzed using appropriate statistical tests of significance to meet the specific objectives of the monitoring program. The statistical analysis and presentation will include, at minimum:

*Statistical characterization (e.g., means, standard deviations, and ranges of water quality parameters) for each site, each stratum, and the Sanctuary as a whole
*Significant differences among strata, including differences among segments and among inshore/offshore strata within the appropriate segments
*Significant trends in water quality parameters within strata and the Sanctuary as a whole
*Graphical and/or statistical analysis of relationships between water quality parameters and water depth and distance from shore or other pollution sources
*Violations of water quality standards and any other indications of polluted conditions

The draft annual report should summarize the objectives, methods, and results of water quality monitoring. The report should interpret the results in relation to the objectives of the monitoring program and the Water Quality Protection Program. The draft annual report will be reviewed by EPA, FDEP, and the Technical Advisory Committee and returned with comments. The principal investigator will address the comments and submit the final annual report with revisions.

2. Coral Reef Evaluation and Monitoring Project for the Florida Keys National Marine Sanctuary

Introduction

The original purpose of this project was to monitor the status and trends of selected coral reefs, patch reefs, and hardbottom in the Florida Keys National Marine Sanctuary as part of the EPA Water Quality Protection Program. From 1996 to 1999, sampling was conducted annually at 40 permanent sites from Key Largo to Key West. In 1999, three additional sites were added to extend the project into the Tortugas North Ecological Reserve and Dry Tortugas National Park to provide baseline data for the Reserve and expand FMRI's historical database for work in the Park. In 2001, the purpose of the project was further expanded to address the goals of the

Coastal Ocean Program's (COP) South Florida Ecosystem Research and Monitoring Program(SFP) to predict (document) impacts of South Florida ecosystem restoration. The project's purpose to detect change was refined in 2002 to potentially determine factors contributing to the documented decline in stony coral cover.

Project Organization

For the first 5 years, The Coral Reef/Hardbottom Monitoring Project (CRMP) was conducted through a grant from the Environmental Protection Agency to the State of Florida's Fish and Wildlife Conservation Commission's Florida Marine Research Institute (FMRI). The Institute contracted with University of Georgia and University of Charleston, S.C. for professional expertise. In FY 00/01, EPA provided half and NOAA provided the remainder of funding. FMRI assumed additional responsibilities and retained only the UGA contract for outside expertise. However, FMRI has retained the services of independent consultants for statistical analyses and microbiology. Since FY01/02, EPA and COP assumed a partnership to provide equal funds for a fully funded expanded Coral Reef Evaluation and Monitoring Project (CREMP).

Project History

After the initial five years, statistical analyses of project data provided for a 33% (49 of 160 original stations) reduction in sampling effort with no reduction in spatial scale which allowed continuation of the project with reduced funding. The station reduction was implemented in summer 2000. During 2001, focus of the project was expanded to include data on bioeroding sponges and distribution of stony coral juveniles. Time gained from station reduction is used to conduct intensified sampling at 9 stations within Value Added Sites (Table 1). Collection of data on stony coral abundance and size classes include juvenile counts. Additional data collection has been designed to ascertain factors (bacterial and/or viral disease, bleaching) contributing to the documented decline in stony coral cover since the inception of the project.

Methods

Underwater sampling is conducted by SCUBA supplemented by NITROX to enhance safety when necessary. FMRI's 37-foot research vessel (R/V Tortugas), outfitted with a portable SCUBA compressor, supports the majority of fieldwork. A detailed description of diving and vessel operations is provided in the Coral/Hardbottom Monitoring Project Quality Assurance Project Plan and the Standard Operating Procedures. All work continues to be conducted under the existing approved Quality Assurance Project Plan. Standard Field Operation Procedures have been revised annually and provided to all CRMP team members. Field SOP's have been revised to incorporate methods for bioerosion, stony coral abundance and recruitment, diseased coral and enterovirus sample collection.

Field Data Collection

Stations were installed during summer/fall 1995 with FKNMS logistical support. In 1996, sampling of only 75% of the stations was completed by summer's end due to adverse weather conditions. OSV Anderson was provided by EPA for a Sanctuary-wide cruise in early October. Remaining stations off Marathon were completed aboard the R/V Tortugas in late October. First annual sampling of all 40 sites was successfully completed during calendar year 1996. All sites have been sampled annually to present.

Station Species Inventory

CRMP has an established protocol. Station species inventory is a census of stony coral presence/absence, selected "disease" or other abnormalities, and *Diadema antillarum*. Data provides species richness of the Scleractinia (stony corals). This method is described in detail in the Coral/Hardbottom Monitoring Project Quality Assurance Project Plan and the Standard Operating Procedures. Task per station requires 20 to 25 minutes. To assure quality and consistency of data, beginning summer 2002, protocol incorporated one senior Principal Investigator and one qualified data collector as frequently as possible.

Video Transects

CRMP has an established protocol. Video sampling is conducted at a fixed 40cm distance from the reef's surface with the video system oriented perpendicularly (0.4 meters above substrate). Paired laser lights, focused to a single point on a reference chain, provide guidance while the camera is slowly moved along the length of each transect. Sampling speed is 4 to 5 meters a minute. Summer 2000 sampling incorporated digital video technology. During 2001, the camera housing was modified with an additional set of laser lights to focus at a fixed 1.5 m from the reef's surface. One additional filming of the station's center (300) transect was conducted at this distance to provide a mosaic overview of the station to facilitate documenting landscape change over time. Task per station is 15 to 20 minutes. Since 2002, the overview transect above the center has been filmed at offshore shallow sites in addition to the Value Added sites.

Bioeroding Sponge Assessment

Data on coral eroding clionid sponges is collected at stations within a 1-m wide belt transect. A PVC pole is held perpendicular to the survey tape, as the observer swims the transect, the location of first intersection of every colony of boring sponge (e.g. C*liona delitrix* or *C. lampa, C. caribboea*) is recorded within a quadrat (5 by 5 cm) which is deployed over the sponge colony. Number of quadrat cells covering the sponge will be recorded. Three transects are sampled at each station. Data is entered into spreadsheet format and provided to the data manger for input into the database. Data will be analyzed to provide an estimate of sponge colony size and distribution and abundance within FKNMS.

Value Added Data Collection

Stony Coral Population Dynamics - Protocol was drafted and field-testing during 2002. This protocol was refined in early 2003. Abundance data for all stony corals (to include a juvenile census and size class distribution) is being collected within a 2x10 meter segment (from the offshore stake to the 10 meter mark) of the SSI station by 1m square quadrats. It is estimated that four dives will be required for sampling at each site.

Disease Condition Tracking - Protocol was drafted and field testing during 2002. Morbid and bleached stony corals, noted during SSI, are documented within the 2 by 10 meter segment of the SSI station. A digital still camera is used to take two photographs (side view with morbid or bleached area and aerial) of affected corals with a clapperboard in the field of view for metadata and scale. Location data is collected to re-locate the individual corals. Estimated time is 2 dives per station with 2 divers.

Rugosity Measurements - The rugosity of the center transect at each of the value added stations is determined using methods described by the NOS-NCCOS National Monitoring Network. These methodologies are employed or modified by habitat type as necessary. Estimated time is 1 dive per station with 2 divers.

Temperature - Small in situ temperature loggers were installed at selected value added sites during 2002 and early 2003. These will be recovered, replaced, and downloaded quarterly. A 15 minute dive is estimated to deploy two units by attaching each instrument to a specified reference stake with cable ties.

Data Reduction

Station Species Inventory - Data from SSI counts is entered, checked, reduced, and analyzed annually following QA/QC procedures.

Video - Initial development of the image analysis software delayed processing and counting of the first year's (1996) video. Post-processing field video continually delayed video image analyses (point counting) and prevented timely analysis of cover data for the first five years. About 25% of the 1999 video was counted prior to the 2000 field season; however, since 2000, all video from that year's summer sampling has been counted prior to the next summer field season.

Annually, the project video is grabbed, converted and analyzed. New techniques of framegrabbing and conversion of images for CD ROMs have been developed for digital video to expedite distribution of the project's video images (CD ROMs) for point counting and to provide timely percent cover analyses. Software (PointCount for Coral Reefs) developed with project funds is used to collect data from digital imagery and has been revised to facilitate efficient image analysis. Ten random points have been determined as optimal for image analysis of the

CREMP video data. Stony corals and other major benthic groups (octocorals, sponges, macro-algae, seagrass, and substrate) are identified and relative percent cover is quantified.

Analyses

All project data are entered into a Microsoft Access database, which facilitates data analyses. After entry into the database, each individual record is checked as part of the QA/QC process. Microsoft Excel is used for preliminary analyses of species richness and frequency of occurrence. In addition, hypothesis testing is performed on the SSI data to determine whether or not there is a difference in the proportion of stations where each species/condition is present. For total stony coral percent cover and individual species, at the station level, hypothesis testing is performed for to compare current year data to all previous years combined. The output of these tests gives the minimum detectable difference that would be deemed significant for a significance level = 0.10 and power = 0.75. At the sanctuary level, non-parametric tests are applied as the data failed the Kolmogorov-Smirnov test for normality. The Kruskal-Wallis H test and the Wilcoxson Rank Sum test are applied to the medians to determine if the data exhibit significant differences.

Proposed Work For FY 2003 - 2004

The field sampling team will consist of a minimum of six staff, preferably seven. We will sample deep offshore, shallow offshore, patch, and hardbottom sites as representative of the reef habitat types in the Florida Keys.

Field Data Collection

At all sites, the CREMP will collect Station Species Inventory (SSI) data [stony coral presence/absence, selected "disease" and bleaching data (including counts of *Diadema antillarum*)] and video as first priority. CREMP work will be supplemented with bioerosion data collection and collection of samples for enterovirus detection at all sites. Aquatic health samples will be collected from a sub-set of diseased corals and octocorals at selected sites. In each geographic locality (upper, middle, and lower keys), the sampling gradient will include a near shore, a Hawk Channel, and an offshore site designated as Value Added sites. Data will be collected at 2 stations at each site.

At Value Added sites, data collection for population dynamics will include stony coral abundance, size class distribution, juvenile counts and tracking of selected diseased corals.

Data Reduction

A summary of the summer 2003 field data (including, SSI, bioerosion and stony coral populations dynamics) will be completed. Framegrabbing and image analysis of the 2003 video data will be completed.

Analyses

Further statistical analyses of all monitoring data (species richness, coral condition and percent cover of major groups including stony corals) to date will be completed to include summer 2003 data.

Reports And Presentations

Quarterly reports have and will be submitted as required. Power point presentations and executive summaries will be provided for the WQPP Technical Advisory and Steering Committees. Executive Summaries, which have been prepared annually, will be submitted with annual summaries of field data to fulfill annual report requirements. A FY 2003 Annual Report consisting of the Executive Summary and summary of all annual data will be submitted. Project staff will address comments by EPA, the Technical Advisory Committee, the Steering committee and NOAA Coastal Ocean Program as requested.

Data Management

As of May 2002, the monitoring data set consists of about 2,100,000 records. The comprehensive data management effort is based at FMRI. Full-time staff are qualified for data entry, summary statistics, and other data management duties.

CREMP data management encompasses the following basic duties: track, concatenate, QA check and compile a master data set of all project data; conduct basic mathematical summaries of annual data; distribute the summaries and master data to principal investigators and project manager on a timely basis; provide summary tables at request of project manager for incorporation into reports as needed and act as liaison between the Monitoring Project, the professional statistical consultants and the Florida Keys National Marine Sanctuary Data Management Workgroup. Staff also liaison with the CAMRA WQPP data management staff.

Data archive and summary distribution is the responsibility of the FKNMS Data Management Workgroup. In December 2001, the data manager transferred all 1996-2001 Station Species Inventory and 1996-2000 video data for inclusion in the second FKNMS Water Quality Protection Program's interactive CD-ROM being produced by FMRI's CAMRA group. In addition, GIS data was provided for computerized mapping of this data. Metadata for both Station Species Inventory and Video data were updated to meet FGDC standards.

Raw data includes copies of all field data sheets, video tapes (Hi-8 for 1996-1999 and digital for 2000-2003), video tape log copies, and a set of annual CD-ROMs. A fire-proof cabinet with water-proof media coolers houses and protects all original video tapes at FMRI. A 20 GB data storage tape is updated weekly with all master data and associated digital products.

Quality Assurance/Quality Control

Internal - All work will be conducted under the existing approved Quality Assurance Project Plan. Refinement of the Standard Operating Procedures is a continuing process. Updated field SOP's will be provided to staff prior to June 2003 work. SOP's for work at Value Added sites were finalized in spring 2003 and incorporated into updated SOP's. Training for disease category recognition is a continuing process. Rapid assessment protocol for bioerosion was developed and bioeroding sponge training was completed in May 2001. Field testing for population dynamics protocols at Value Added stations was completed in March 2003.

External - We will continue to consult with EPA Region IV QA/QC officer and FMRI QA/QC officer on issues involving QA/QC. The following colleagues serve the CREMP as a review panel:

Dr. Robert K. Clarke, Plymouth Marine Laboratories
Dr. Terry Done, Australian Institute of Marine Science
Dr. Steven Gittings, Flower Garden Banks Marine Sanctuary
Dr. Caroline Rogers, U.S. Biological Survey
Dr. Deborah L. Santavy, U.S. Environmental Protection Agency

Dr. Chris Tsokos and Dr. George Yanev have been retained to conduct further analyses of the monitoring data generated to date.

3. Seagrass Monitoring Project for the Florida Keys National Marine Sanctuary

Objectives

The general objective of seagrass monitoring is to measure the status and trends of seagrass communities to evaluate progress toward protecting and restoring the living marine resources of the Sanctuary. Specific objectives are as follows:

1. To provide data needed to make unbiased, statistically rigorous statements about the status and temporal trends of seagrass communities in the Sanctuary as a whole and within defined strata

2. To provide a framework for testing hypothesized pollutant fate/effect relationships through process-oriented research and monitoring

Monitoring is defined here as the continued observation of seagrass communities to determine spatial and temporal variability. Monitoring involves systematic, long-term data collection and analysis to measure the status of these communities and to detect changes over time. Detecting such changes can focus research on determining the cause, can prompt management decisions for corrective action, and can be used to evaluate the success of corrective action.

Overview

Seagrass monitoring will involve *in situ* measurements of population and community level characteristics. Seagrass communities will be monitored using a stratified random design based on the Sanctuary segmentation framework (Klein and Orlando 1994). During the first 7 years of this project, three sets of sites have been monitored:

- **Level I sites (shoot morphometrics and *Thalassia* productivity)**
  Randomly chosen, permanent sites will be sampled quarterly to estimate *Thalassia* productivity and analyze shoot morphometrics. This will allow a comparison of the two approaches to characterizing seagrass community status as a function of the information obtained and the costs of sampling and analysis.

- **Level II sites (shoot morphometrics)**
  Wider geographic coverage will be obtained by monitoring additional, randomly chosen sites for shoot morphometrics alone. Sampling will occur annually, with new sites chosen each year.

- **Level III sites (cover-abundance)**
  Even wider geographic coverage will be accomplished by using a rapid, semi-quantitative approach to characterizing seagrass community status through measurement of cover-abundance at randomly chosen sites. Sampling will occur annually, with new sites chosen each year.

The mix of site types was intended to monitor trends through quarterly sampling at a few permanent locations (Level I sites) and to annually characterize the broader seagrass population through less intensive, one-time sampling at more locations (Level II and III sites). We feel that we have adequately characterized the spatial distribution of benthic habitats in the FKNMS at this time, so in FY 2001 Level II and Level III sites were not monitored. We have determined that the rate of change on these benthic communities occurs on a time scale longer than yearly, so continued yearly broad scale monitoring will not cost-effectively detect trends. However, we propose the collection of Level II and Level III information at some point in the future.

Sampling methods are comparable to those being used to monitor seagrass in Florida Bay and Biscayne Bay. Seagrass communities in these areas are being monitored by researchers from Everglades National Park, Florida International University, the University of Virginia, FMRI, EPA/EMAP, and the Miami-Dade County Department of Environmental Management. The approach and methods described in this program have been developed with the collaboration of the primary researchers involved in the ongoing programs.

Monitoring Locations

Monitoring locations have been chosen to be compatible with other monitoring programs being

conducted by Everglades National Park, Florida International University, FMRI, and EPA/EMAP). Level I sites were located to coincide with water quality monitoring stations.

Level I Sites

Level I sites are located in Segments 4, 5, 6, 7, and 9. There are six sites in segments 4, 6 and 7, and seven sites in segments 5 and 9. In Segments 5, 7, and 9 (Atlantic side of the Keys), two sites will be located in each of the following strata: nearshore, Hawk Channel, and offshore. No further stratification is planned within the other segments.

Because it is advantageous to co-locate biological and water quality monitoring sites, and because seagrass is nearly ubiquitous in the Sanctuary, all Level I (permanent) sites will be located at or near water quality stations. Within each stratum, one of the several water quality stations will be picked at random and the seagrass site will be located at the water quality station or in the nearest seagrass bed on a random heading. Randomization of Level I sites is assured by the process used to position water quality stations.

Level II Sites

Level II sites will be located in all segments except number 8. There will be six sites each in Segments 4, 5, 6, 7, and 9; nine sites each in Segments 1 and 2; and three sites in Segment 3. Level II sites will be located randomly within each segment using the EMAP grid. In Segments 5, 7, and 9 (Atlantic side of Keys), the sites will be located within each of the following strata: nearshore, Hawk Channel, and offshore. No further stratification is planned within the other segments.

Level III Sites

Level III sites will be located in all segments except number 8. There will be 10 sites in Segment 3 and 30 sites in each of the others. Level III sites will be located randomly within each segment using the EMAP grid. In Segments 5, 7, and 9, sites will be located in each of the following strata: nearshore, Hawk Channel, and offshore. No further stratification is planned within the other segments.

Planned Sampling for FY2005 and FY 2006

Sampling at the Level 1 sites will continue on a quarterly basis. The resampling of the Level 3 sites originally sampled in 1997 will be completed in FY 2004. In FY 2005, Level 2 and Level 3 sites sampled in FY 1998 will be resampled, and in FY 2006, sites sampled in FY 1999 will be resampled.

In order to describe the spatial extent and pattern in the benthic communities, monitoring sites were selected across the extent of the monitoring area. This monitoring program was designed

to assess status and trends in seagrass communities across the entire extent of the Florida Keys National Marine Sanctuary, a 9,000 km^2 area of ocean surrounding the Florida Keys. It was recognized early in the monitoring program that the expansive shallow marine habitats immediately to the north of the Sanctuary on the southwest Florida Shelf also were important for determining the status of seagrass communities within the Sanctuary itself, so the monitoring program was extended to cover these additional 8,000 km^2 as well (Figure 1). It is a goal of the program to describe spatial pattern in the indicators of interest, hence it is important to sample the entire region. A distributed, stratified-random algorithm was used to choose sampling sites for synoptic mapping across the region of interest. The locations for each site were chosen by laying a probability-based grid over the area of interest, and then randomly choosing a location within each grid cell. This method allows sampling locations to be spaced quasi-evenly across the landscape while still maintaining the assumptions required for a random sample, i.e. all locations had an equal probability of being sampled. In each of the first 5 years of the monitoring program (1996-2000), the same arrangement of grid cells was employed, but new random points were selected within each cell each year. This allows for the development of synoptic maps of measured indicators during each monitoring year, as well as a combined data set of quasi-evenly spaced random points collected over 5 years. The original monitoring plan called for revisiting the first year's sites during the sixth year, the second year's site during the seventh year, etc - so that trends in the resource over a 5-year interval could be tested with *n* pair-wise comparisons for 5 years in a row. However, because of the slow rate of change observed at the permanent monitoring stations, it was decided to delay the beginning of the resampling until FY 2003, when the stations first surveyed in FY 1996 were revisited.

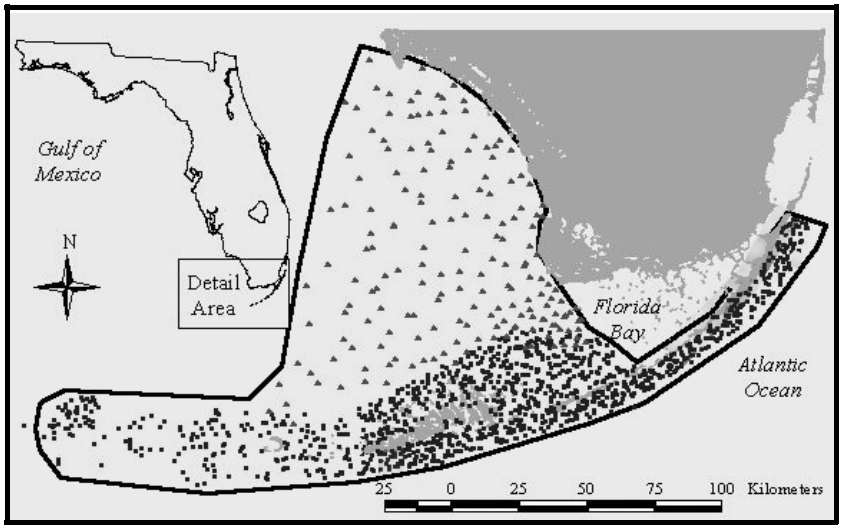

Figure 1. Study area. Heavy black line denotes 17,000 km2 study area, dots indicate 1200 synoptic sampling points surveyed during 1996-2000.

The locations of the Level 2 and Level 3 sites to be sampled in FY 2005 and FY 2006 are available upon request. These sites are distributed throughout the FKNMS and across the broad, shallow portion of the southwest Florida Shelf to the north of the FKNMS (Figure 1).

Parameters and Methods

Monitoring will provide measures of population and community level characteristics in the

seagrass community, including seagrass cover, density, growth rate, standing crop, productivity, and seagrass elemental content. The parameters to be measured and the sampling frequency and number of sites for each level of effort are listed in tabular form and are available upon request. Level I sites will be sampled quarterly.

Sampling techniques to be used in the monitoring program are based on the methodology primarily used by Fourqurean, Zieman, and Durako in Florida Bay to monitor seagrass die-off. The techniques include both rapid, qualitative assessments and more labor-intensive quantitative methods:

- Shoot morphometrics will be analyzed from randomly selected shoots at each Level I and Level II site.

- Cover-abundance of seagrass will be estimated at each Level I, Level II, and Level III site.

- Seagrass elemental content (Carbon, Nitrogen and Phosphorus) will be determined for all seagrass species present at Level I and Level II sites, following the methods described in Fourqurean et al. (1992).

- Assessment of physiological status of *Thalassia testudinum* using PAM fluorometry, following the methods described in Beer et al. 1998, Beer and Bjork 2000, and Ralph and Dennison 1998.

Seagrass Physiological Status

In conjunction with the productivity measurements, we will also assess the status of *Thalassia testudinum* by measuring the rate of electron transport (ETR) in the light-harvesting organs of the seagrasses using a submersible PAM fluorometer. This rate is proportional to photosynthesis; and the relationship between photosynthesis and productivity will be assessed as a gage of the health of the plants. We will determine the light-photosynthesis curve for 3 leaf sections from 3 representative short shoots from each productivity quadrat. We will generate the following parameters for each site:

- Mean and variance in light-saturated rate of photosynthesis (P_{max})
- Mean and variance in the quantum yield of photosynthesis ( )
- Mean and variance in the half-saturation coefficient (I_k) of photosynthesis
- Mean and variance in light-saturated rate of electron transport (P_{max})
- Mean and variance in the quantum yield for electron transport ( )
- Mean and variance in the half-saturation coefficient (I_k) of electron transport

Shoot Morphometrics

At Level I sites, shoot morphometrics will be measured on all seagrass short shoots harvested from the productivity quadrats during the summer quarter sampling. These shoot samples will be analyzed for:

- no. of species
- no. of short shoots per species
- no. of blades per short shoot
- no. of new shoots, fruits, and flowers
- no. of leaf scars and no. of leaves per short shoot
- shoot age (no. of leaf scars + no. of standing leaves)
- plastochrone interval
- canopy height

Seagrass Elemental Content

Five samples of representative short shoots of each species will be collected at each Level I site for determination of C, N and P content. The number of shoots collected for each sample is a function of species, with 5 *Thalassia testudinum*, 10 *Syringodium filiforme*, and 15 *Halodule wrightii* shoots being collected. These shoots will be selected arbitrarily, and collected in a manner that ensures sampling of complete shoots. These will be stored in a plastic bag on ice and transported back to the laboratory. Leaves will be separated from the shoots, and cleaned of epiphytes by gently scraping with a sharp blade. All blades from a sample will be pooled, rinsed in tap water, and dried to constant weight at 60 C. Dried samples will be homogenized in a mortar and pestle or a mill. Samples will then be stored over dessicant until they are analyzed.

Carbon and nitrogen content will be determined for duplicate subsamples from each sample, using an automated, combustion technique. Phosphorus content will be determined in duplicate using a dry-oxidation, acid-hydrolysis procedure (details of methods in Fourqurean et al. 1992).

Other Samples/Observations

Qualitative cover-abundance observations will be recorded to allow cross-comparison of data. Each Level I site will be surveyed each quarter for seagrass and macroalgal abundance using the Braun-Blanquet cover-abundance scale. Depending on the community type and macrophyte density, a grid or a transect will be set up at each site, and cover-abundance in six to ten 0.25 m^2 quadrats located randomly within the grid or along the transect will be assessed according to the following scale:

5 any number, with cover of more than 75% of the quadrat
4 any number, with 50 to 75% cover
3 any number, with 25 to 50% cover

2 any number, with 5-25% cover
1 numerous, but less than 5% cover, or scattered with up to 5% cover
+ few, with small cover (assigned a value of 0.5)
r solitary with small cover (assigned a value of 0.1)

The upper four scale values (5, 4, 3, 2) refer only to cover. The lower three scales are primarily estimates of abundance, i.e. the number of individuals per species. Frequency of occurrence, abundance, and density information for a species within a transect will be calculated using the following formulas:

- Frequency = number of occupied quadrats/total number of quadrats
- Abundance = sum of Braun-Blanquet scale values/number of occupied quadrats
- Density = sum of Braun-Blanquet scale values/total number of quadrats

The presence/absence of fleshy epiphytic algae, calcareous epiphytic algae, and macroalgae will also be noted.

Quality Assurance/Quality Control

A Quality Assurance Program for seagrass monitoring has been approved by EPA. This program will be ammended to cover C:N:P determinations. In accordance with EPA policy, monitoring will adhere to existing rules and regulations governing QA/QC procedures as described in EPA guidance documents. The principal investigators will consult with the EPA Region IV QA/QC Officer on any issues involving QA/QC matters.

Data Management

The principal investigators will develop and maintain protocols and procedures under a data management program to ensure that the data generated are accessible to potential users in a timely manner. All original and ancillary data produced under this project will be generated, processed, stored, and archived in a manner that provides detailed documentation of the procedures used at all stages of data collection, reduction, processing, analysis, and storage.

Under a cooperative agreement with EPA, FMRI developed a data management plan and prototype data management system for the monitoring and research programs. The principal investigators will work with FMRI to identify priority data needs, define data entry formats and QA/QC protocols, and resolve data management conventions and issues (e.g., station nomenclature and codes, parameter codes, the geographic datum, missing number codes, error flags).

Reporting

The principal investigators will produce a site map, quarterly data reports, and an annual report.

The principal investigators will be responsible for ensuring the results are compiled and the complete data set is submitted in a timely fashion to FMRI for inclusion into the Sanctuary database.

Site Map and Coordinates

Upon completion of the first survey, the principal investigators will produce a summary map of the monitoring network with a listing of sites, GPS coordinates, and water depths.

Quarterly Data Reports

Upon completion of the analysis of samples from each quarterly survey, the principal investigators will produce a statistical summary of the data in a logical format based on the sampling design. The statistical summary will include calculated averages, sample variances, ranges, and number of samples. When appropriate, the principal investigators will provide the summaries in a graphical format. The principal investigators will submit a data and narrative report documenting the results of each quarterly survey. The data report will include the raw data and statistical summaries in hard copy and on disk. The principal investigators will evaluate all data in accordance with the data quality objectives developed in the Work/Quality Assurance Project Plan.

Annual Report

The principal investigators will produce statistical summaries of the data collected at each seagrass monitoring site to be incorporated in an annual report. All data will be evaluated in relation to the data quality objectives developed in the Work/Quality Assurance Project Plan. The data will be analyzed using appropriate statistical tests of significance to meet the specific objectives of the monitoring program. The statistical analysis and presentation will include, at minimum:

- Statistical characterization (e.g., means, standard deviations, and ranges of parameters measured) of each site, each geographic segment, and the Sanctuary as a whole
- Significant differences among geographic segments
- (In future years) Significant trends within geographic segments and the Sanctuary as a whole
- Relationships between seagrass and water quality parameters
- Any indications of unusual conditions possibly indicative of pollution

The draft annual report should summarize the objectives, methods, and results of seagrass

monitoring. The report should interpret the results in relation to the objectives of the monitoring program and the Water Quality Protection Program. The draft annual report will be reviewed by EPA, FDEP, and the Technical Advisory Committee and returned with comments. The principal investigators will address the comments and submit the final annual report with revisions.

4. Data Management Program for the Water Quality Protection of the Florida Keys National Marine Sanctuary

Project Purpose

Goal - The goal of this project is to provide a data integration system that takes into account the varying levels of data produced by individual monitoring projects and the needs of both managers and researchers. In order to accurately incorporate the different levels of scientific data produced, the data integration system is comprised of two components: data archives and data integration.

Data Archives - The data archives component encompasses both raw and synthesized data. These data sets will be stored in a centralized location in the original formats presented by the individual projects. No data manipulation including formatting, standardizing, or merging will be done for, or within, this component of the data integration system. Access to these data, in their original form and content, will be provided upon request and approval from the Sanctuary Manager to researchers, managers, and the general public.

Data Integration - Result data, both tabular and geospatial, are to be integrated for incorporation into a geographic information system to facilitate further analysis by researchers and managers. The result data that are to be contained within the database integration system will be documented with project level metadata as well as attribute or parameter level metadata. Integration summaries will also be available within the integrated database.

Work Plan

Background and Approach

The Florida Keys National Marine Sanctuary was created with the signing of the Florida Keys National Marine Sanctuary and Protection Act on
November 16, 1990. Included in the Sanctuary are 2900 square nautical miles of nearshore waters extending from just south of Miami to the Dry Tortugas. The 1990 Act directed the Environmental Protection Agency (EPA) and the State of Florida, in consultation with National Oceanic and Atmospheric Administration (NOAA), to develop a Water Quality Protection Program (WQPP) for the Sanctuary. This is the first marine sanctuary required to have a WQPP.

The purpose of the WQPP is to recommend priority corrective actions and compliance schedules addressing point and nonpoint sources of pollution to restore and maintain the chemical, physical, and biological integrity of the Sanctuary. The 1990 Act also requires development of a comprehensive water quality monitoring and research program and was delivered to NOAA in May 1993. In addition to the 1990 Act, Congress passed the National Marine Sanctuaries Program Amendment Act of 1992. Section 2209 of this Act directs the EPA and the State of Florida to implement the WQPP.

The EPA and the State of Florida have developed an implementation plan for the monitoring, research, and data integration programs. Management priorities, available funds, and estimated costs were considered in developing each of the programs. The monitoring program is divided into three on-going projects: water quality, seagrass, and coral reef/hardbottom. The research program encompasses a wide variety of geographically specific projects, all of a short-term duration. The data integration program combines and integrates the data produced by the other programs.

The WQPP document specifically recommends the establishment of a regional database and data management system for recording the biological, physical, and chemical results from the comprehensive monitoring and research programs. Therefore, in July 1993, the EPA issued a cooperative agreement (#X994346-93-0) to the Florida Department of Natural Resources (now FWCC) Marine Research Institute for the development of a data management plan and prototype data management system. Since then, significant progress has been made in achieving these goals. The data management plan was completed in December 1995. This document outlined a holistic approach to data management enveloping all the various FKNMS WQPP projects and addressed the issues of data entry, access, storage, documentation, and security. The various aspects of the data management system were explored and implemented using this holistic approach in tandem with both other EPA efforts and the development of the individual monitoring project's efforts. EPA's STORET modernization effort was designated to be a keystone upon which to build a data entry and access tool for researchers, managers and the public that incorporated levels of security and included imbedded documentation from the organization level down to specific sampling results. Development of the new and improved STORET database, STORET X, originally was scheduled for completion in 1996. However, there were unexpected setbacks that pushed final version delivery back twelve months to December 1997. Although a prototype of the final version was released in June 1996, several severe restrictions to the database and its graphical user interface front end prohibit its use as an interim FKNMS WQPP database.

Coinciding with the development problems of STORET X was the disparity of the data collection and synthesis by the monitoring projects. It was determined that two levels of data (results and synthesized) would be accumulated with both needing incorporation into the resultant database integration system. It was also discovered that although a holistic approach addressed all aspects of data management, it was not practical technically, physically, or fiscally. In order to effectively manage the two data streams, a new practical approach to data

management needed to be derived from the original holistically proposed data management system.

Therefore, to accommodate these findings, a data archives system is in place that allows researchers to archive their data for long-term storage and enable managers to point to a centralized location where data have been deposited. To effectively manage all forms of data, a data integration system is being developed. This system uses a GIS tool, such as ArcView, to view and query these datasets along with associated metadata and ancillary information.

This integration system focuses on the cross-utilization of the various data sets produced by the individual FKNMS WQPP projects through standardized formats. As data are entered into the data integration system, it will become part of a large FKNMS resource and be made available through the publication of a FKNMS WQPP CD-ROM.

Currently, principle investigators are evaluating their monitoring and analysis efforts for the next five-year life cycle of the WQPP. Their multiple focus is data collection, data analysis and implementation. Results from this evaluation effort may shift the requirements for data management. FMRI, in cooperation with the principle investigators and EPA, will modify the data integration system to accommodate the Program. FMRI will monitor the development of Modernized STORET and associated interface software and all data associated with the WQPP's comprehensive monitoring will be incorporated into STORET, when feasible.

Work Tasks

Obtain and Archive Raw Data Sets - FMRI will continue to work with each of the individual monitoring and research projects to obtain copies of their raw data sets for incorporation into the archive portion of the FKNMS data integration system.

Obtain Synthesized Data Sets - FMRI will continue to work with each of the individual monitoring and research projects to obtain copies of their synthesized data sets for incorporation into both the archives and CD-ROM distribution of the FKNMS data integration system.

Obtain Geographic and Ancillary Data Sets - FMRI will continue to build upon a continuing effort to acquire, automate and manage ancillary geo-spatial data to complement the FKNMS data integration system.

Design, Produce and Distribute a CD-ROM that Houses Each Long-Term Monitoring Project's Interpreted Data, Metadata, and Ancillary Information - FMRI will make all possible efforts to integrate the information into one robust geographic information system (GIS) database. FMRI will work with each of the principle investigators to make sure their data and analyses are correctly represented to reflect their research focus. FMRI will evaluate and adjust the data management outputs according to the focus of the five-year cycle of the WQPP.

Continue to Explore New Technologies and Methodologies to Integrate Each Principle Investigator's Synthesized Data - FMRI will continue to explore new technologies that facilitate the integration of the diverse data being collected and interpreted in the FKNMS WQPP. These technologies may include HTML programming, multimedia, and the Internet. FMRI will also monitor the development of Modernized STORET and its associated interface software.

Provide Technical Support - FMRI will provide technical support to each principle investigator and FKNMS personnel for all issues associated with the data management and integration system.

Reports And Presentations

Quarterly reports have and will be submitted. Power point presentations and executive summaries will be provided for the WQPP Technical Advisory and Steering Committees as necessary and when requested by the EPA project officer. Annual Reports will be submitted. Project staff will address comments by EPA, the Technical Advisory Committee, the Steering committee and NOAA Coastal Ocean Program as requested.